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THE EFFECT OF THE SUBSTRATUM ON THE MORPHOLOGY AND PHYSIOLOGY OF A PYTHIUM SPECIES FROM SAFFLOWER

F. R. Harper

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ABSTRACT

Various factors affected growth, fruiting, sporangial germination and pathogenicity of a Pythium sp. isolated from diseased safflower roots. The fungus grew at temperatures from 5° to 29° C., produced sporangia and oospores from 10° to 25° C. and was pathogenic to safflower from 5° to nearly 30° C. Growth at 29° C. declined with time on agar media containing certain plant extracts but not on agar media containing inorganic salts plus various nitrogen sources and sucrose. A number of nitrogen sources and carbohydrates affected growth, sporulation and size of sporangia of the fungus. There was no apparent relationship between rate of growth and fruiting of Pythium sp. on the nitrogen sources and carbohydrates on which the fungus was grown. Some factor found in tap water and soil extract but not in distilled water induced direct germination of sporangia and a factor found in an extract of safflower roots plus pretreatment at -10° C. induced indirect germination of sporangia. Safflower variety N. 3 was less susceptible to Pythium sp. than N. 9 at all temperatures and ages studied. Pythium sp. attacked alfalfa, sugar beet, carrot, alsike clover and sweet clover in addition to safflower, but did not attack a number of cereals and grasses, onion, pea, bean, cucumber, flax and turnip.

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THE EFFECT OF THE SUBSTRATUM ON THE MORPHOLOGY AND PHYSIOLOGY OF A *PYTHIUM* SPECIES FROM SAFFLOWER

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THE EFFECT OF THE SUBSTRATUM ON THE MORPHOLOGY AND PHYSIOLOGY OF A PYTHIUM SPECIES FROM SAFFLOWER

INTRODUCTION

Safflower (Carthamus tinctorius L.) is an important oilseed crop that has been grown experimentally in southern Alberta since 1942 (32). The results to date indicate that late maturity and root rot damage are the major limiting factors to commercial production of the crop in this area. Severe root rot in certain varieties was first noted in irrigated experimental plots at Lethbridge in 1949 (7). Isolations from diseased roots yielded a number of colonies of an unidentified species of Pythium. Pure cultures of several isolates of this fungus were highly pathogenic to safflower seedlings in greenhouse experiments. Further studies were started since field observations indicated that Pythium root rot could be very destructive to susceptible varieties of safflower.

A thorough study of the causal fungus was first necessary. Information on the influence of certain factors on the morphology and growth of Pythium sp. would be useful to future studies on the taxonomy of the genus. Also, the effect of various factors on the disease would be of value to the plant pathologist and plant breeder in formulating control measures. With these two ideas in mind the present study was undertaken to determine the effect of temperature and a number of substrates on the growth, fruiting, germination of sporangia and pathogenicity of the Pythium sp. from safflower.

ROOT ROT OF SAFFLOWER

Root rot damage has been noted in safflower plots on irrigated land at Lethbridge and Rosemary, Alberta (Fig. 1). No evidence of root rot has been observed on susceptible varieties of safflower grown on non-irrigated land.

Symptoms of Pythium root rot in safflower seedlings were a dull green color of the cotyledons followed by collapse of the whole plant. The hypocotyl exhibited bluish-black or brown lesions. Older plants were stunted and exhibited a progressive wilting or drying up of the leaves, commencing with those nearest the base of the stem. The stem remained erect. Severely affected plants were easily pulled up as lateral root development was almost completely eliminated, leaving only the main root still attached to the stem. Fig. 2 depicts varying degrees of Pythium sp. damage to safflower seedlings.

Hyphae and sporangia of Pythium sp. were observed in the cells of the hypocotyl of diseased safflower seedlings. Oospores were noted in partially disintegrated roots from plants killed by the pathogen.

No other Pythium has been reported causing root rot of safflower in the field. Ramos (36) found that Pythium debaryanum Hesse could cause damping-off of safflower seedlings in the greenhouse. Phytophthora drechsleri Tucker has been reported by Erwin (14) to cause a root rot of safflower in Nebraska and California.

A number of varieties of safflower were reported resistant to Pythium root rot by Cormack and Harper (8). Classen (5) reported certain of these varieties reacted similarly to Phytophthora root rot.

FIG. 1. Damage to safflower varieties caused by Pythium sp. The four-row plot in the centre is N. 9 ; those at the left and right are N. 472 and Indian respectively.

FIG. 2. Varying degrees of damage to safflower seedlings by Pythium sp. Plants from left to right are healthy, slightly infected, severely infected and dead.

THE PATHOGEN

The following is a brief description of Pythium sp. from safflower based on the writer's observations.

Hyphae branched with rounded tips, 2 to 8 μ (usually 6 μ) in diameter, mainly intramatrical, rarely septate, forming clavate appressoria under certain conditions. Sporangia extra- and intramatrical, terminal or intercalary, spherical, oval, oblong, obovoid, pyriform or allantoid, 8 to 56 μ wide by 8 to 110 μ long, with walls varying in thickness from 0.5 to 3 μ, germinating commonly by 1 to 8 germ tubes or under certain conditions a vesicle giving rise to zoospores 6 to 8 μ in diameter when encysted. Oogonia spherical, smooth, terminal rarely intercalary. Antheridia 1 to 4, usually 1 to 2 per oogonium, monoclinous or diclinous, when monoclinous not arising adjacent to the oogonium. Antheridia and frequently a portion of antheridial hyphae making close contact with the oogonial wall. Oospores spherical to subspherical, aplerotic, sometimes almost filling oogonium, wall 1.5 to 2 μ thick, containing a single reserve globule and refringent body. Oospore germination not observed.

Fig. 3 and Fig. 4 illustrate some of the characteristics of Pythium sp. from safflower.

This fungus is related to those species of Pythium which have spheroidal, non-proliferous sporangia, smooth oogonia, monoclinous or diclinous antheridia and smooth aplerotic oospores. The species included in this group are P. polymorphon Sideris, P. ultimum Trow, P. paroecandrum Drechsler, P. splendens Braun, P. vexans de Bary and P. debaryanum Hesse (33).

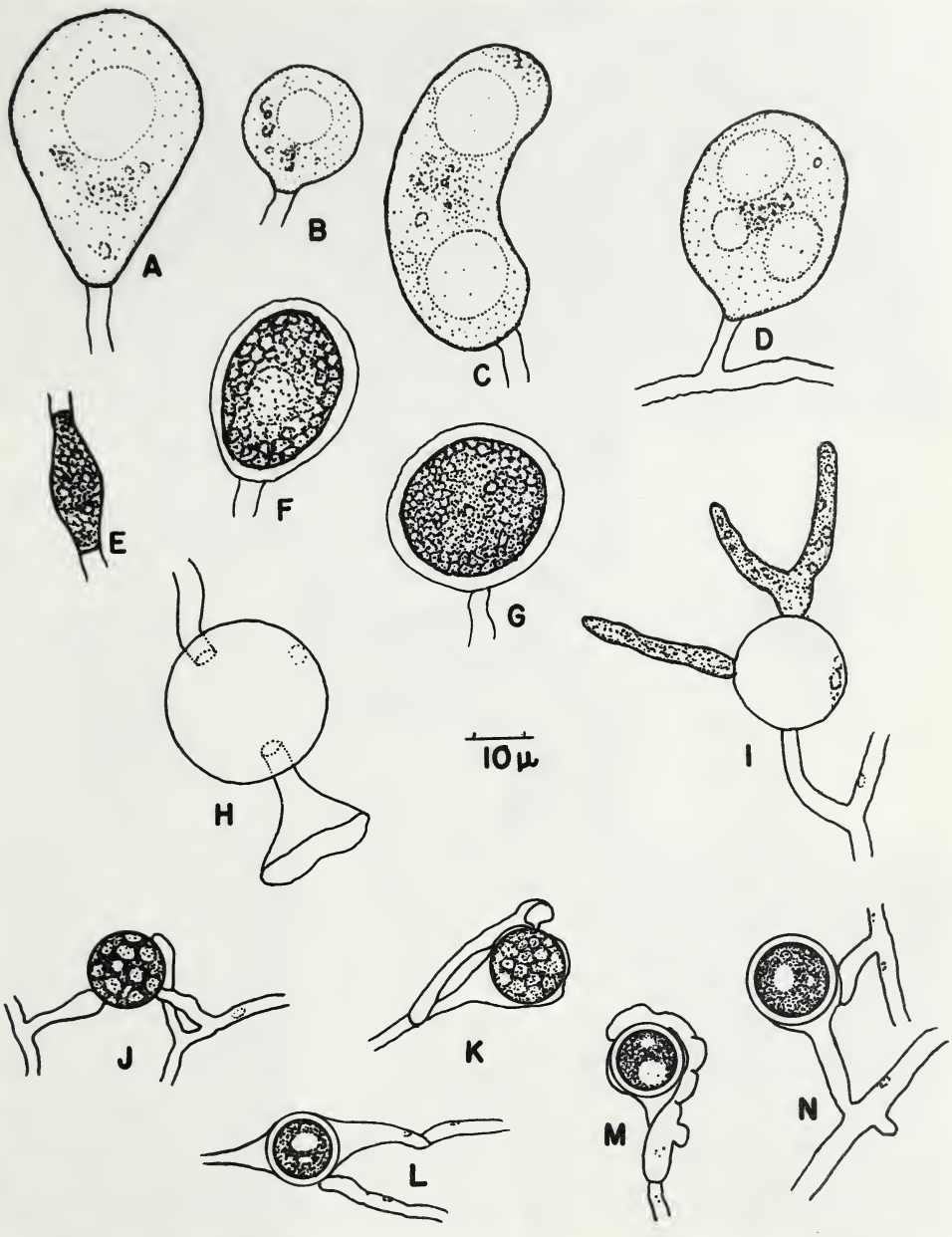

FIG. 3. Pythium sp. from safflower. A-G. Sporangia. H. Empty sporangium and remains of vesicle. I. Sporangium germinating by germ tubes. J, K. Oogonia and antheridia. L-N. Oospores with oogonial and antheridial remains. X 750.

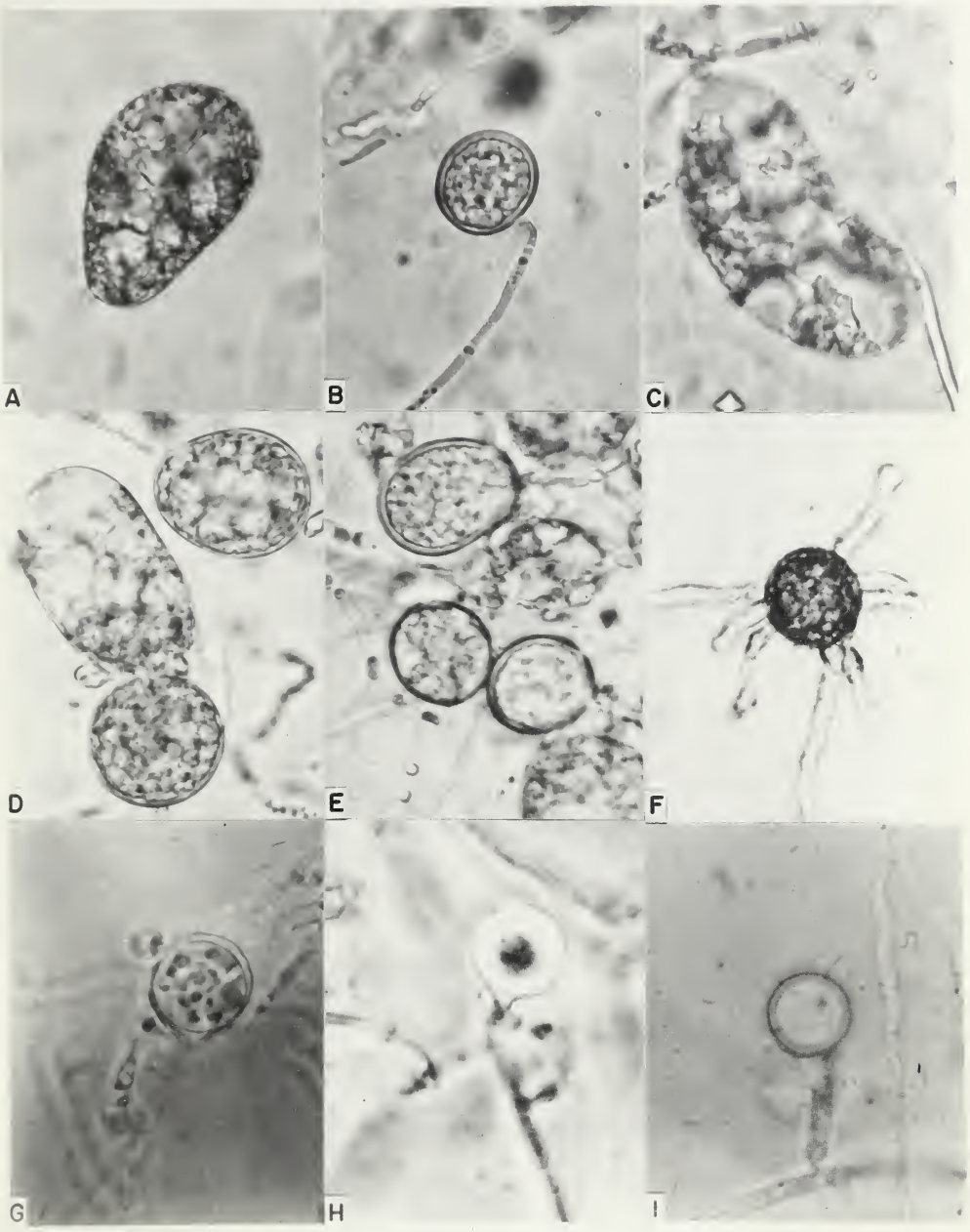

FIG. 4. Asexual and sexual stages of Pythium sp. A-E, sporangia. F. Germinating sporangium. G. Oogonium and antheridium. H, I. Oospores. X800.

The six Pythium species listed above have higher optimum and maximum temperatures for growth than the Pythium sp. from safflower (33). Pythium sp. exhibited greater variation in size and shape of sporangia than the six species it most closely resembled. None of the six species was reported to produce thick-walled sporangia similar to those of Pythium sp. (2, 33, 43). Broad contact of the antheridia with the oogonium separates Pythium sp. from P. debaryanum, P. ultimum and P. polymorphon. The presence of thick-walled oospores differentiates the safflower pathogen from P. debaryanum, P. paroecandrum and P. vexans all of which have thin-walled oospores (33). Radiate growth of the safflower pathogen on agar media is similar only to P. vexans of the group mentioned above (33).

No direct comparison of the differences between cultures of Pythium sp. from safflower and other closely related Pythium species has yet been undertaken to determine whether this fungus varies sufficiently from other species to consider it a new species.

MATERIALS AND METHODS

Isolation Method

Isolations from lesioned roots were made by surface sterilizing small portions of lesioned roots with 0.1 percent mercuric chloride for 45 seconds. Following this the root portions were thoroughly washed in sterile distilled water, transferred aseptically to petri dishes of potato dextrose agar and incubated at room temperature. Colonies growing from the

root pieces were recorded at four and ten days.

Isolates

An isolate of Pythium sp., designated isolate A, was used throughout these studies. This isolate was obtained in 1949 from a safflower root in an experimental planting near Lethbridge. Two other isolates were utilized in some of the studies reported here. Isolate B was procured in 1952 from safflower grown at Rosemary, Alberta and isolate C was obtained in 1954 from a diseased safflower plant grown at Lethbridge. The three isolates differed little in appearance on culture media and in sporangial development. Oogonial and antheridial characteristics of isolates A and C were similar. Difficulties in obtaining sufficient sexual fruiting of isolate B hampered comparison of its antheridial, oogonial and oospore characteristics with those of the other two isolates.

The three isolates were maintained on potato dextrose agar from the time of isolation.

Media

A number of semisynthetic media were used in the present studies. Formulae for preparation of these media are given in Appendix 1. Granulated agar from the same lot was used in all media. Media were sterilized in an autoclave at 15 to 18 p.s.i. for 15 minutes. Hydrogen ion concentration was adjusted to pH 6 by the addition of 0.1 N. sodium hydroxide or 0.1 N. hydrochloric acid before sterilization. Glassware was cleaned with a solution of sodium dichromate and sulphuric acid and rinsed with distilled water before use.

Transfer Material

Uniform material for transfer to petri dishes and growth tubes was obtained by cutting 4 mm. discs from a four- to seven-day old petri dish culture of the fungus. These discs were placed aseptically at the centre of the petri dishes or at one end of the growth tubes.

Growth Determinations

Growth determinations were made from cultures of the fungus in growth tubes patterned after those described by Ryan, Beadle and Tatum (38). The tubes were made from "Pyrex" tubing 12 mm. in outside diameter and 30 cm. long. Each tube was bent upward at an angle of 45 degrees at a point 4 cm. from each end. Each tube contained 5 ml. of agar medium.

To start growth, the tubes were incubated for 24 hours at room temperature before they were transferred to the temperature of the experiment. The limit of mycelial growth at the end of each successive 24 hour period was recorded by marking a line on the outside of the tube opposite the margin of growth. Mean linear growth was determined by averaging the growth in millimeters attained on the fourth, fifth and sixth days of incubation by quadruplicate cultures of the fungus. Mean daily linear growth was the average growth in millimeters recorded by quadruplicate cultures on the day specified.

Sporulation Assessment

Fourteen-day old petri dish cultures containing 15 ml. of medium were used to assess sporulation. Samples of medium and

associated fungus structures, 1 by 4 mm. in surface dimensions, were transferred from the petri dish to a drop of lactofuchsin (4) on a glass microscope slide and orientated so that the former surface was on one side. A coverslip was then placed over the sample and pressure was applied to flatten the mount. The resultant preparation was a somewhat distorted section through the medium. This procedure allowed examination of fruiting at the surface and within the matrix. The use of lactofuchsin as a mountant enabled the preparation of semipermanent slides in minimum time. The lactofuchsin stained the protoplasm red but did not stain the walls of hyphae or sporangia. Sporulation was estimated numerically by examining two samples from each petri dish with a microscope, using a magnification of 300 diameters. The estimates used were as follows:

Rating class	Average number of fruiting bodies per field (based on three fields)
0	none
1	fewer than 1
2	1 to 5
3	6 to 50
4	over 50

Sporangia were most numerous at the upper surface of the medium and oospores were usually found near the lower surface of the medium. Each estimate of sporulation was based on the examination of three fields in the area where sporangia or oospores were most numerous. If no fruiting was encountered in the three fields a systematic search of the whole mount was made under low magnification to determine

whether any sporangia or oospores were present. At least two plates were examined for each treatment reported.

Measurement

All measurements of sporangia and oospores were made with the aid of a microprojector. Widths quoted in these studies were based on the maximum width of each organ measured. Average widths are based on measurement of approximately 100 sporangia or oospores unless otherwise stated.

Pathogenicity Tests

Six-inch unglazed clay pots containing steam sterilized 3:1 mixture of loam and sand, were used in all pathogenicity tests with the exception of those carried out at controlled soil temperatures.

Material for infesting the soil with the safflower pathogen was prepared by adding the contents of two seven-day old petri dish cultures of the organism to 400 ml. distilled water in a Waring blendor and triturating this material for two minutes. Each pot to be infested received 100 ml. of the resultant suspension. Control pots received an equivalent amount of distilled water. In certain cases a second series of control pots received a suspension of agar medium in distilled water. No differences were noted between the controls receiving distilled water and those receiving the suspension of agar medium.

The degree of damage in each pot was assessed on the basis of survival to the termination of the experiment. Percent survival was calculated as follows:

$$\text{Percent survival} = \frac{\text{number of surviving plants}}{\text{number of seeds sown}} \times 100$$

Seed of varieties N. 3 and N. 9 was used in all pathogenicity tests against safflower. Germination of N. 3 and N. 9 was 83 percent and 72 percent respectively. A separate lot of N. 9 used in the temperature studies showed 58 percent germination. Unless otherwise stated, 10 seeds of each variety were sown in each pot at a depth of 4 cm.

FACTORS INFLUENCING THE GROWTH OF PYTHIUM SP.

The effect of temperature on the growth of many fungi in culture has been studied (15, 26, 33, 39). Fawcett (15) reported, in his studies with four citrus pathogens, that on agar plates there was a tendency for growth rate to decrease with time if the temperature was above that at which maximum growth occurred. Middleton (33) in his studies on the effect of temperature on growth of Pythium spp. confined his measurement of growth on agar media to a single definite time period, thus decrease in growth rate with time was not reported. Much of the other work on temperature in relation to growth of fungi was based on a single time interval either on agar media or in liquid culture. The effect of various media on the growth of fungi at near-maximum temperature has evidently not been studied. Fawcett (15) used a single medium in his temperature studies.

Steinberg (44, 45), Perlman (35), Foster (16), Lilly and Barnett (28) and Hawker (20), have reviewed the literature on the effect of various nutrients on growth of fungi in artificial culture.

Rosenbaum (38) found that certain natural media affected the growth rate of various Phytophthora spp. Brancato and Goldring (1) found that certain concentrations of sucrose, glucose, glycerol, ethylene glycol and sodium chloride in the medium supported greater growth of species of Penicillium, Aspergillus and Geotrichum than either higher or lower concentrations. Hacskaylo, Lilly and Barnett (19) found that nitrate nitrogen was utilized slowly by a few of the twenty-five fungi studied, although ammonium nitrate and asparagine supported good growth of all twenty-five fungi. No Phycomycetes were included in the studies by Hacskaylo et al. Lopatecki and Newton (31) reported that four Phytophthora spp. differed in their ability to utilize ammonium and nitrate salts. They found that P. cactorum could utilize ammonium but not nitrate and P. megasperma could utilize nitrate but not ammonium as a nitrogen source. P. parasitica and P. erythroseptica were able to use both ammonium and nitrate to supply their nitrogen needs. Saksena, Jain and Jafri (41) found that thirteen species of Pythium could utilize sodium nitrate, ammonium chloride, glycine, alanine, asparagine and glutamic acid as nitrogen sources. Twelve of the thirteen species could utilize urea and five of the thirteen could utilize acetamide as nitrogen sources. Variations in the composition and concentration of carbon source were shown to affect the growth of Sclerotium rolfsii (Johnson and Joham 24). Lilly and Barnett (29) reported that a number of species of fungi differed in their ability to utilize d- and l-arabinose.

Temperature

Isolates A, B and C of Pythium sp. growing on cornmeal agar were placed at temperatures of 0°, 5°, 10°, 15°, 20°, 25° and 29° C. Fig. 5 depicts the mean linear growth of the three isolates at the above temperatures.

Growth was appressed at all temperatures. Mycelial development on the surface of the agar was more sparse at 5° and 10° C. than at 15°, 20° and 25° C. No measurable growth occurred at 0° C. in six days but growth commenced the first day following transfer to room temperature. Similarly growth occurred only the first day at 29° C. but resumed when the cultures were subsequently transferred to room temperature.

The growth rate of the three isolates of Pythium sp. tended to differ. Isolate B showed a slower rate of growth than isolates A and C at all temperatures. Isolates A and C differed noticeably from each other in growth rate only at 20° and 25° C. The three isolates showed no differences in their ability to initiate growth at extreme temperatures as none grew at 0° C. or after the first day at 29° C.

Medium

Experiments similar to the one described above were carried out with Pythium isolate A using potato dextrose, potato sucrose, lima bean sucrose and sucrose nitrate agar media. Table 1 shows the growth of isolate A on the various media at temperatures from 0° to 29° C.

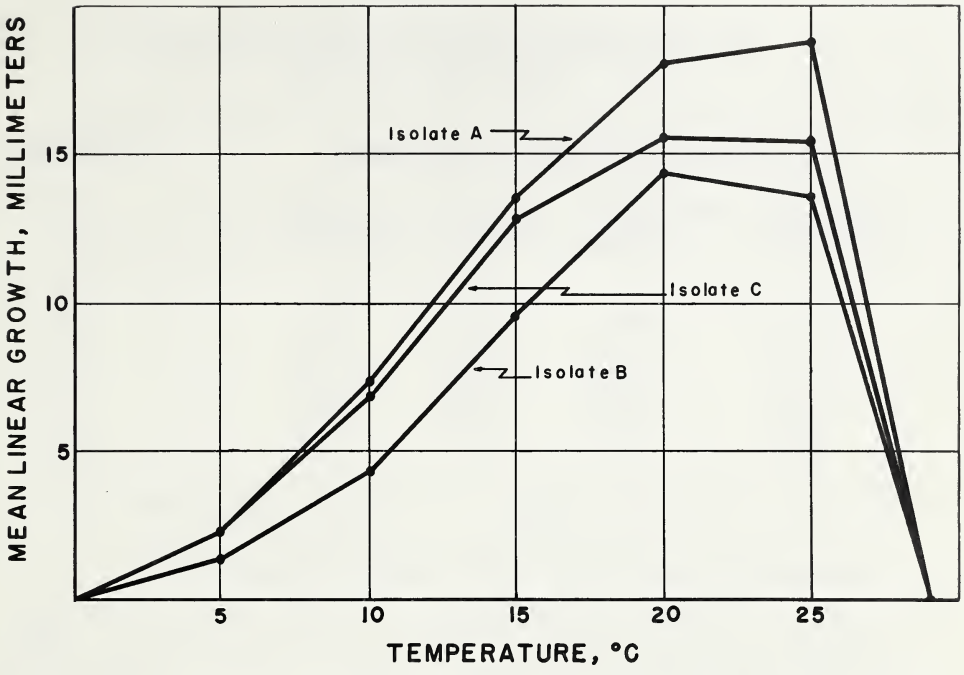

FIG. 5. Effect of temperature on growth of Pythium sp. on cornmeal agar.

TABLE 1

Influence of media on growth of Pythium sp. at various temperatures.

Temperature, ° C.	Mean linear growth on agar media, mm.*			
	Potato dextrose	Potato sucrose	Lima bean sucrose	Sucrose nitrate
0	0	0	0	0
5	3.2	4.7	5.4	2.8
10	11.7	12.4	14.1	11.6
15	21.4	22.8	24.1	15.9
20	28.6	29.8	33.5	19.7
25	29.5	30.6	31.5	19.2
29	0	0	0	6.1

* F (media) = 9.07 L.S.D. = 1.6 mm.

Similar growth studies were carried out with isolates B and C on potato dextrose and sucrose nitrate agars. Results were similar to those in Table 1 except that differences in growth rates of the three isolates shown in Fig. 5 were maintained.

Table 1 shows that the growth rate of Pythium sp. on potato dextrose and potato sucrose agars is the same. Growth rate of Pythium sp. at 15°, 20° and 25° C. on sucrose nitrate agar, is significantly lower than on potato dextrose agar. Growth of Pythium sp. on lima bean agar is significantly higher than on potato dextrose agar at all temperatures from 5° to 25° C.

Some growth occurred on all media at 29° C. but only on sucrose nitrate agar did it continue to the termination of the experiment. Table 2 summarizes the growth of Pythium sp. isolate A on the four media at 29° C.

TABLE 2

Mean daily linear growth of Pythium sp. on various media at 29° C.

Agar medium	Mean daily linear growth, mm.				
	1st day	2nd day	3rd day	4th day	5th day
Potato dextrose	3.3	0	0	0	0
Potato sucrose	8.8	4.0	0.5*	0	0
Lima bean sucrose	9.8	5.8	2.8*	0	0
Sucrose nitrate	9.3	5.3	6.0	6.3	6.0

* One or more replicates showed no growth the third day.

It is difficult to explain the results shown in Table 2. The sucrose nitrate medium as it supplied only inorganic salts was more likely to be deficient in a growth requirement than the plant extract media which supplied more complex nutrients. As media containing both dextrose and sucrose showed the same type of decrease in growth rate with time, carbon source was ruled out as a causal factor. The various plant extract media presumably supplied an organic form of nitrogen while the sucrose nitrate medium supplied sodium and calcium nitrate. Titration of the various media with hydrochloric acid and sodium hydroxide indicated slight differences in buffer capacity among the media. In view of these differences experiments were set up to determine the effect of various nitrogen sources and buffer capacities on growth of Pythium sp. at near-maximum temperatures.

Nitrogen source

Sucrose nitrate agar was modified by the substitution of equivalent nitrogen from a number of nitrogen sources for the sodium and calcium nitrate present in the original medium. Sodium chloride and calcium chloride were added to maintain the sodium and calcium level in the medium. Table 3 shows the effect of the various nitrogen compounds on the growth of isolate A at 25° and 29° C.

TABLE 3

Growth of Pythium sp. on various nitrogen sources

Nitrogen source	Mean linear growth, mm.	
	25° C.	29° C.
Sodium and calcium nitrate	19.0	5.4
Ammonium nitrate	19.6	5.5
Ammonium chloride	19.0	5.3
Ammonium acetate	12.5	4.0
d-Glycine	14.2	2.8
l-Cysteine	1	0
dl-Asparagine	13.2	6.5
Casein	21.0	2.4
Gelatin	19.1	3.3

Pythium sp. grew well on all of the nitrogen sources tested with the exception of cysteine. Where similar nitrogen sources were used these results agree with those of Saksena et al, (41).

At 25° C., protein and inorganic nitrogen sources, with the exception of ammonium acetate, supported a higher growth rate of Pythium sp. than the amino acids. At 29° C. no generalization appears to be possible.

Growth of Pythium sp. at 29° C. continued to the termination of the experiment on all media except the one containing cysteine which supported no measurable growth.

Buffer Capacity

Pythium sp. isolate A was grown on sucrose nitrate agar which had been modified by the addition of various amounts of 0.1 M. potassium phosphate. Table 4 shows the mean linear growth of the organism on the various levels of this buffer at temperatures of 25° and 29° C.

TABLE 4

Influence of various amounts of 0.1 M. potassium phosphate on growth of Pythium sp.

Potassium phosphate, * ml./ l.	Mean linear growth, mm.	
	25° C. **	29° C.
12.5	19.8	3.8
25.0	20.6	3.6
50.0	15.3	3.6
100.0	15.8	2.9

* 5 parts KH_2PO_4 to 1 part K_2HPO_4
** L.S.D. = 1.9 mm.

There was a significant decrease in growth rate of Pythium sp. at 25° C. on the two highest concentrations of potassium phosphate. All cultures in these treatments continued growth to the termination of the experiment.

Carbon source

Attempts were made to grow Pythium sp. on various carbohydrates. The carbohydrates listed in Table 5 were incorporated into the sucrose nitrate medium in place of sucrose. Each carbohydrate was supplied at 20 gm. per litre of medium. Table 5 shows the mean linear growth of Pythium sp. isolate A at 25° C. on media containing the various sugars.

TABLE 5

Effect of various carbohydrates on growth of Pythium sp. at 25° C.

Carbohydrate	Mean linear growth, mm.
Monosaccharides	
D-xylose	1
D-arabinose	1
L-arabinose	10.4
D-glucose	12.7
D-manose	4.8
D-fructose	3.4
Oligosaccharides	
Sucrose	16.2
Lactose	17.4
Maltose	25.6
Cellobiose	16.8
Raffinose hydrate	17.5
Polysaccharides	
Dextrin	14.7
Starch (soluble)	15.4
Xylan	19.9
Inulin	16.7
Cellulose	16.4
Pectin (citrus)	0

The growth rate of Pythium sp. was higher on the oligosaccharides and polysaccharides, except pectin, than on the monosaccharides. Hirst and Jones (21) reported that pectin from different sources and different stages of plant growth differed in composition. Pectin from sources other than the one used may support growth of Pythium sp.

Differences in the ability of Pythium sp. to utilize the various monosaccharides for growth points to the lack of specific enzymes for utilization of certain of these sugars. Differences in the utilization of D- and L-arabinose agree with the findings of Lilly and Barnett (29) that various fungi may utilize one of these isomers and not the other.

Growth of Pythium sp. on maltose and sucrose was equal to the sum of the growth rates on the monosaccharide moities of these sugars, (glucose plus glucose for maltose, and glucose plus fructose for sucrose). Growth of Pythium sp. on cellobiose and raffinose did not show the same relationship to their monosaccharide moities as did maltose and sucrose.

FACTORS AFFECTING SPORULATION OF PYTHIUM SP.

Classification in the genus Pythium is based on the characteristics of sporangia, oogonia, antheridia and oospores. In artificial culture many species of Pythium do not fruit readily, although a number of workers found media and treatments that induced formation of sporangia and oogonia by the species they were studying. Potato dextrose agar, lima bean agar, cornmeal agar, tap water agar,

grated carrot agar, wheat agar and oatmeal agar have all been used successfully by various workers to induce fruiting by many Pythium and Phytophthora species, (2, 11, 23, 26, 33, 37, 40, 49). Middleton (33) found that certain Pythium species which did not form sporangia on agar media, formed these organs when grown on pea broth and then transferred to running tap water. Vanterpool (49) added a few root-tips from wheat seedlings to tap water agar to induce oogonial formation by the Pythium spp. with which he was working. Rands and Dopp (37) found the addition of a water extract of humus to cornmeal agar supported the formation of oogonia by a number of Pythium spp. Saksena (40) found that light had no effect, peptone retarded, and codliver oil accelerated the formation of sexual organs of the four Pythium species he studied.

The use of media containing plant material, plant extracts or other complex organic materials, has evidently been a standard practice in inducing fruiting by various Pythium species.

Medium

Cultures of Pythium sp. growing on a number of agar media were examined for the presence of sporangia and oospores. Table 6 records the effect of these media on sporulation.

Isolates A, B, and C of Pythium sp. showed similar reactions on the media listed in Table 6. All media supported production of sporangia by Pythium sp. but only lima bean sucrose agar and wheat-root agar were favourable for oospore development. On the wheat root agar oospores of the organism were formed only

within the cells of the wheat roots, which made observation of the various stages of oospore development difficult.

TABLE 6

The influence of various media on sporulation of Pythium sp.

Agar medium	Sporulation *	
	Sporangia	Oospores
Potato dextrose	+	-
Potato sucrose	+	-
Lima bean sucrose	+	+
Tap water	+	-
Wheat root	+	+
Turnip sucrose	+	-
Sucrose nitrate	+	-
Cornmeal	+	-
Safflower sucrose nitrate, N. 3	+	-
Safflower sucrose nitrate, N. 9	+	-
Safflower sucrose, N. 3	+	-
Safflower sucrose, N. 9	+	-

* (+) - reproductive organs present; (-) - no reproductive organs present.

Temperature

As Pythium sp. produced both sporangia and oogonia on lima bean sucrose agar, this medium was used to study the effect of temperature on sporulation. Isolates A, B, and C of Pythium sp. were grown on lima bean sucrose agar, in controlled temperature chambers set at 5°, 10°, 15°, 20° and 25° C., for 14 days. Two cultures grown at each temperature were examined for the presence of sporangia and oospores. Table 7 gives the ratings observed.

There was some variation in size of sporangia produced at the various temperatures. In view of this widths of sporangia and oospores of the three isolates were measured. Table 8

gives the range and mean widths of sporangia and oospores for the three isolates of Pythium sp. at the various temperatures listed in Table 7.

TABLE 7

Influence of temperature on sporulation of Pythium sp.

Temperature, ° C.	Rating at 14 days, 0-4		
	Isol. A.	Isol. B.	Isol. C.
Sporangia			
5	0	1	0
10	3	3	4
15	4	3	4
20	4	4	4
25	4	4	4
Oospores			
5	0	0	0
10	1	0	1
15	2	1	2
20	2	1	2
25	2	1	2

TABLE 8

Effect of temperature on width of sporangia and oospores of Pythium sp.

Temperature, ° C.	Width, μ					
	Isolate A		Isolate B		Isolate C	
	Range	Mean	Range	Mean	Range	Mean
Sporangia						
10	10-20	16.7	13-26	18.2	12-19	15.9
15	15-30	21.1	16-32	23.8	18-34	25.4
20	14-34	23.1	16-35	22.8	18-29	23.4
25	10-47	27.1	15-30	21.6	18-35	23.4
Oospores						
10	-	-	-	-	-	-
15	14-21	16.5*	-	-	12-18	14.2*
20	13-20	15.8*	-	-	11-18	13.9
25	12-21	16.5*	-	-	11-18	13.7

* Estimate based on fewer than 100 oospores.

Pythium sp. isolates A and C differed little in the number of sporangia produced at the various temperatures. Isolate B produced a few sporangia at a lower temperature than isolates A and C and also produced fewer sporangia at 10 and 15° C. than the other two isolates.

The three isolates produced smaller sporangia at 10° C. than at the other three temperatures. At 15° and 20° C. the three isolates differed little in the size of sporangia produced. At 25° isolates B and C produced sporangia similar in size to those produced at 15° and 20° but isolate A produced larger sporangia.

Oospore production by isolates A and C was similar at all temperatures, except that isolate C produced oospores which were approximately two microns smaller than those of isolate A. Isolate B produced too few oospores at any temperature to serve as an estimate of spore size.

As the three Pythium sp. isolates fruited more readily at 20° and 25° C. and the average size of sporangia of the three isolates appeared to be more uniform at 20° C. than at 25° C. further studies on sporulation were carried out at 20° C.

Nitrogen Source

Attempts were made to determine the effect of certain nitrogen sources on fruiting by Pythium sp. The nitrogen sources were substituted, on an equivalent nitrogen basis, for the sodium and calcium nitrate in the sucrose nitrate agar. Sodium chloride and calcium chloride were added to avoid any change in

the sodium and calcium levels of the medium. Table 9 shows the effect of various nitrogen sources on production and size of sporangia of Pythium sp. isolate A.

TABLE 9

Effect of nitrogen source on number and size of sporangia of Pythium sp.

Nitrogen source	No. present 0-4	Size, μ		
		Length	Width	Average Width
Sodium and calcium nitrate	3	16- 82	16-43	28.8
Ammonium nitrate	3	14-110	17-56	26.1
Ammonium chloride	1	-	-	-
Ammonium acetate	3	13- 44	13-30	18.5
d-Glycine	4	15- 67	15-46	24.5
dl-Asparagine	4	13- 42	16-35	20.5
l-Cysteine	0	-	-	-
Casein	3	16- 34	16-27	20.3
Gelatin	4	16-52	16-43	24.6

No oospores were found in samples from any of the media listed in Table 9.

All the nitrogen sources tested, with the exception of ammonium chloride and cysteine supported good sporangial formation by Pythium sp. As ammonium nitrogen supplied by ammonium acetate, and amino nitrogen supplied by glycine and asparagine were able to support the formation of sporangia by the fungus the lack of fruiting on ammonium chloride and cysteine was probably due to some factor other than the nitrogen portion of these two nitrogen sources.

The number of sporangia produced by Pythium sp. on a particular nitrogen source had no bearing on the size of those produced.

Media containing nitrate, glycine and gelatin supported greater variation and larger sized sporangia than the media containing ammonium acetate, asparagine and casein.

Carbon Source

The effect of a number of carbohydrates on fruiting of Pythium sp. was determined. Fruiting was assessed on sucrose nitrate agar containing various other carbohydrates in place of sucrose. Each carbohydrate was supplied at the rate of 20 gm. per litre of medium. Table 10 shows the effect of various carbohydrates on the number and size of sporangia produced by Pythium sp. isolate A.

TABLE 10

Effect of carbohydrate on size and number of sporangia of Pythium sp.

Carbohydrate	No. present, 0-4	Size, μ		
		Length	Width	Average Width
Monosaccharides				
D-xylose	1	-	-	-
D-arabinose	1	13-36	11-34	23.9*
L-arabinose	1	13-38	8-31	23.1*
D-glucose	3	16-71	16-47	30.5
D-manose	2	10-36	10-35	23.0
D-fructose	3	17-82	15-49	25.7
Oligosaccharides				
Sucrose	3	15-75	15-45	27.3
Lactose	3	10-51	10-36	19.5
Maltose	4	15-61	15-35	25.1
Cellobiose	4	16-53	16-35	24.4
Raffinose hydrate	3	14-47	14-26	20.3
Polysaccharides				
Dextrin	4	16-65	16-32	25.9
Starch (soluble)	4	17-51	17-40	26.6
Xylan	1	-	-	-
Inulin	2	11-28	11-28	21.7
Cellulose	2	8-28	8-28	16.4
Pectin (citrus)	0	(no growth)		

* Measurements based on fewer than 100 sporangia.

Glucose and fructose were the only monossaccharides to support good sporangial formation by Pythium sp. All the oligosaccharides and the nutrient polysaccharides dextrin and starch supported good fruiting, with maltose, cellobiose, dextrin and starch supporting ratings of 4. The remaining polysaccharides produced few sporangia.

With the exception of lactose, the carbohydrates which supported sporangial ratings of 3 and 4 also supported larger sporangia which were more variable in size than the carbohydrates which supported sporangial ratings of 1 and 2.

A few oospores of Pythium sp. were found in two of eight samples taken from plates containing inulin as a carbohydrate source. No oospores were encountered in samples from any of the other carbohydrate sources listed in Table 10. The scarcity of oospores in the samples in which they were found and absence of oospores in other samples from inulin-containing media suggested that some factor other than the carbohydrate source determined the formation of these spores.

FACTORS AFFECTING SPORE GERMINATION OF PYTHIUM SP.

Fungus spores require certain conditions for germination. Gottlieb (18) stated that all fungus spores require water, either as a liquid or vapor, for normal germination. Yarwood (50) and Brodie and Neufeld (3) found that spores of Erysiphe graminis tritici and Erysiphe polygoni can germinate at humidities of 0 to 100 percent. Clayton (6) and Durrell (13) found that spores of Sclerotinia fructicola and Puccinia coronata required free water for germination.

Water alone is insufficient for the germination of many fungus spores. A number of fungi require the presence of other nutrients such as carbohydrates or leachates from plant material before they will germinate. According to Noble (34) Urocystis tritici spores germinated in the presence of wheat seeds but not in water alone. Uppal (47) found the presence of carbohydrates necessary for inducing a high percentage germination of Phytophthora infestans sporangia.

Most Pythium and Phytophthora species produce sporangia which may germinate either by the production of germ tubes (direct germination) or zoospores (indirect germination). Sporangia of Phytophthora infestans and Phytophthora palmivora were found by Uppal (48) to require oxygen and high temperatures for direct germination. At low temperatures the sporangia of these two fungi germinated indirectly even in the absence of oxygen.

The zoospore method of germination is often difficult to induce in Pythium. Drechsler (11) induced the formation of zoospores in a number of Pythium species by transferring cornmeal agar blocks containing the fungus to dishes and then adding water to, but not above, the level of the agar surface. Middleton (33) found that mycelial mats of Pythium grown in pea broth and then transferred to running tap water produced sporangia which germinated by zoospore formation. Middleton also found that certain Pythium species were induced to produce sporangia which germinated by zoospore production when lima bean agar blocks containing the fungus

were irrigated with tap water following the addition of soil or decaying organic matter.

Germination of oospores of many Pythium species has rarely been reported. Drechsler (12) was able to induce the germination of oospores of Pythium debaryanum and P. ultimum by transferring three-month old cornmeal agar plate cultures of these fungi to a thin layer of water in petri dishes.

Germination of Sporangia

A number of attempts were made to induce sporangia of Pythium sp. to germinate. Attempts to induce indirect germination of sporangia by continual irrigation of these organs on the surface of lima bean sucrose, potato dextrose and sucrose nitrate agar cultures with distilled and tap water were unsuccessful. The method described by Drechsler (11) was also tried, with distilled and tap water, an aqueous filtrate of soil and a water extract from ground safflower roots. The results of these studies are reported in Table 11.

TABLE 11.

Effect of various treatments on type of sporangial germination of Pythium sp. at 20 to 22° C.

Liquid	Pretreatment	
	None	-10° C. *
Distilled water	none	none
Tap water	direct	direct
Soil extract	direct	direct
Safflower root extract	direct	indirect

* Dishes placed at -10° C. for 1-2 hours then transferred to room temperature.

These preliminary results indicate that some factor other than water is necessary for direct germination of Pythium sp. sporangia. For indirect germination, a period of below freezing temperature appears to be necessary.

FACTORS AFFECTING PATHOGENICITY OF PYTHIUM SP.

Root rot may be affected by the action of the environment on the host, on the pathogen or on both simultaneously. Dickson (9) found that wheat seedlings were attacked by Gibberella saubinetii more severely at high temperatures than at lower temperatures, while corn seedlings were attacked by the same parasite more severely at low temperatures than at high temperatures. Dickson and Holbert (10) suggested that severe damage to corn seedlings by Gibberella saubinetii was correlated with high pentosan and low suberin and soluble carbohydrate content of the root cell walls. Leach (25) found the relative growth of the host and pathogen at various temperatures gave a definite indication of seedling damage to sugar beets, pea, watermelon and spinach by Pythium ultimum, Rhizoctonia solani, Phoma betae and Aphanomyces cochlioides. Erwin (14) found in his studies on Phytophthora root rot of safflower that temperatures of 25° to 30° C. were favourable for severe root rot, while temperatures below 17° C. prevented root rot damage.

The age of the plant when attacked by a fungal pathogen may have an effect on the severity of the disease. Thomas (46) found that fewer safflower plants were killed by Phytophthora drechsleri as the age of the plants, when the soil was infested, increased.

The medium on which the fungus is grown may have an effect on the pathogenicity of the fungus to its host, although Linnasalmi (30) found that growth of Pythium debaryanum and P. ultimum on various agar media did not affect their pathogenicity to various glasshouse crops when these fungi were later introduced into the soil.

The pathogenicity of a fungus to hosts other than the one from which it was isolated has been given considerable attention. Rhizoctonia solani has been studied frequently with regard to strains varying in pathogenicity to various hosts. Sanford (42) found that a number of isolates of R. solani isolated from potato were not able to attack sugar beets. Houston (22) also working with R. solani, found that morphological characteristics of various cultures were more accurate in predicting the pathogenicity of the culture than were the hosts from which they were isolated. Leonian (27) found that isolates obtained from a single sporangium of Phytophthora omnivora differed widely in both morphology and pathogenicity. Linnasalmi (30) found a number of isolates of Pythium debaryanum and P. ultimum able to cause damping-off of cabbage, cauliflower, cucumber and tomato

seedlings but the isolates used did not show any specialization to the host from which they were isolated.

Age of Plant

Safflower varieties N. 3 and N. 9 were grown in sterilized soil which was infested with the safflower pathogen at the time of seeding, 10 days or 20 days after seeding. The roots of surviving plants were examined for root rot damage ten and twenty days after infesting the soil. Table 12 shows the percentage survival in the various treatments twenty days after infesting the soil with Pythium sp. isolate A.

TABLE 12

Effect of age of plant on survival of two safflower varieties in soil infested with Pythium sp.

Age when infested, days	Percent survival, 20 days			
	N. 3		N. 9	
	Infested	Control	Infested	Control
0	22.5	63.8	0	80.0
10	67.5	76.3	0	73.8
20	60.0	85.0	0	82.5

The results in Table 12 indicate that N. 3 was susceptible to severe damage by the pathogen only in the early seedling stage while N. 9 was equally susceptible to severe damage throughout the period of time under study.

The roots of almost every surviving plant removed from Pythium sp. infested soil had some root lesions.

In two similar experiments conducted during May and June, the fungus had little effect on either variety of safflower. This may have been due to killing of the pathogen by high soil temperatures, or higher light intensities increasing the resistance of the host to the pathogen.

Temperature

Glazed clay crocks, six inches in diameter and 12 inches deep, were filled to within four inches of the top with a three-to-one loam-sand mixture and sterilized. Each crock was seeded with ten seeds of each safflower variety. Eight crocks were placed in each of six Wisconsin type controlled soil temperature tanks set at 5°, 10°, 15°, 20°, 25° and 30° C. Four of the crocks at each temperature were infested with Pythium sp. and the other four served as controls. Fig. 6 depicts the survival of the two safflower varieties when transferred to the various temperatures immediately after seeding and infesting the crocks. Fig. 7 depicts the survival of the two varieties when they were grown on a greenhouse bench for two weeks before they were transferred to temperature tanks and infested. The results in Figs. 6 and 7 are based on seven-week old plants. Fig. 8 shows the damage to 17-day old safflower plants three days after infesting the soil with Pythium sp.

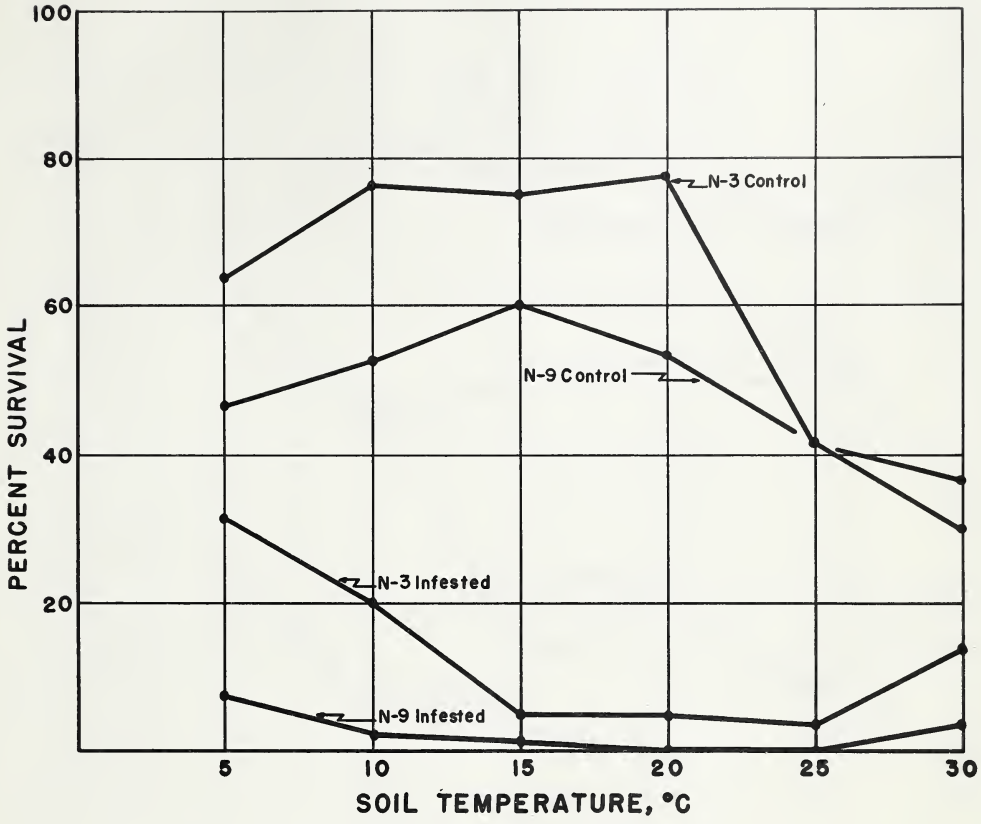

FIG. 6. Effect of temperature on survival of safflower seedlings in soil infested with Pythium sp. at seeding.

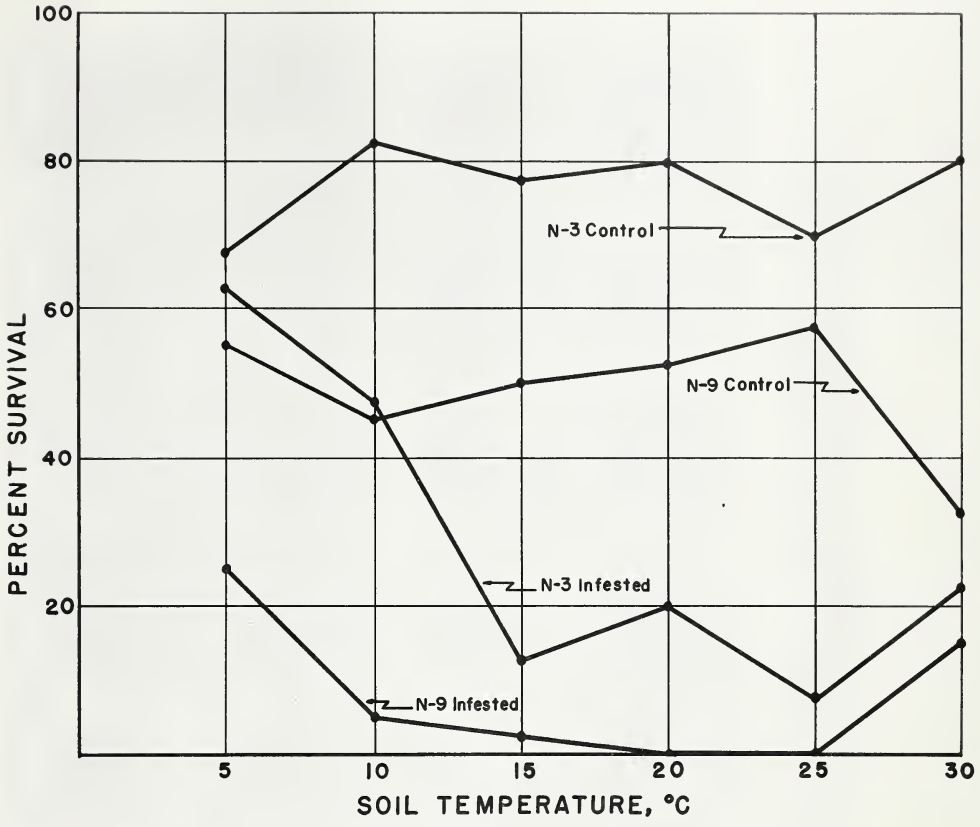

FIG. 7. Effect of temperature on survival of safflower seedlings in soil infested with Pythium sp. two weeks after seeding.

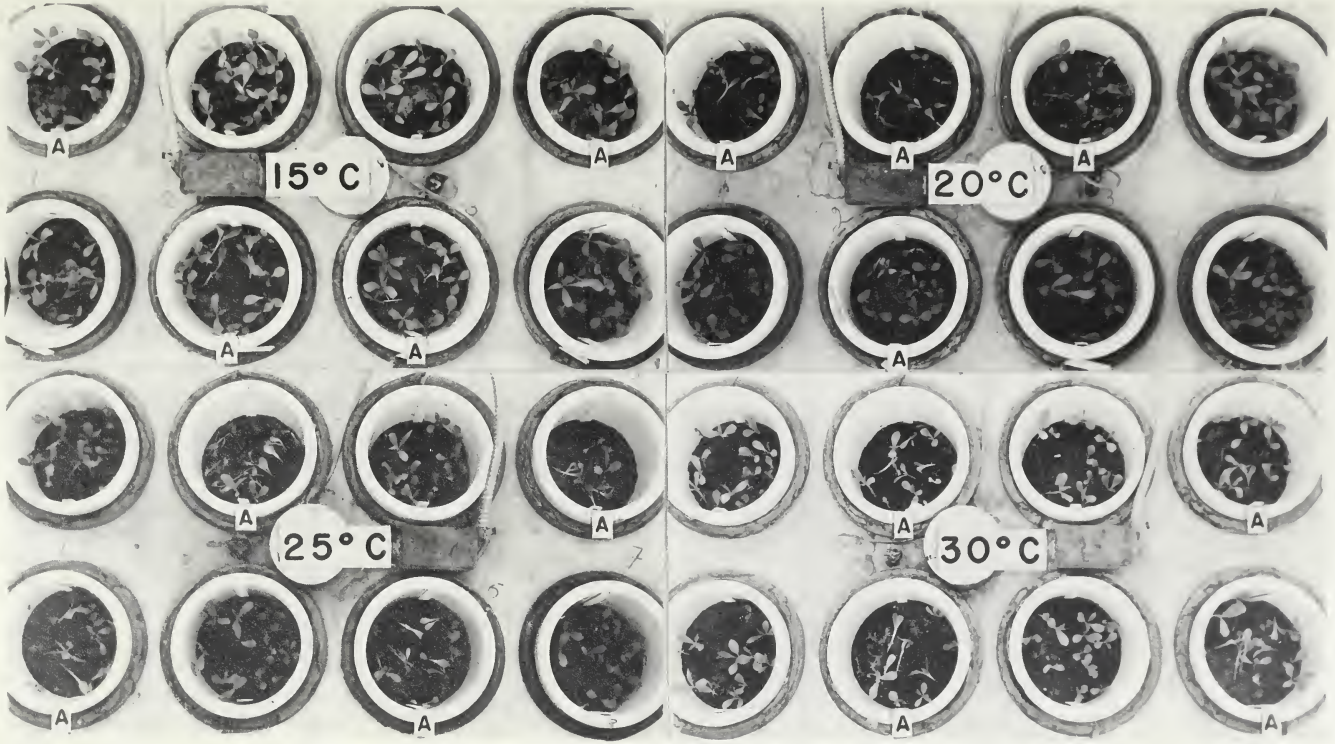

FIG. 8. Survival of safflower seedlings at various temperatures in Pythium sp. infested soil. The left half of each pot contains N. 9 and the right half contains N. 3. Pots marked A were infested three days previously with Pythium sp. Unmarked pots are controls

The results shown in Figs. 6 and 7 indicated that variety N. 3 was more resistant than N. 9 to Pythium sp. attack at all temperatures. The results also indicated that when soil infestation was delayed two weeks N. 3 was more resistant than when soil was infested at the time of seeding. The increased resistance of N. 3 is more noticeable at 5° and 10° C. than at the other temperatures. When soil infestation was delayed N. 9 showed a slight increase in survival at 5° C. but practically none at temperatures from 10° to 25° C.

Survival of plants in the 30° C. temperature tank appeared to be associated with position of the plants in the pots. Plants growing near the edge of the infested pots survived to the termination of the experiment while those near the centre of the same pots were killed, (see Fig. 8). It was found that at a depth of one inch the temperature at the centre of the pots was approximately 28° C. while at the outer edge of the pots the temperature was between 29° and 29.3° C. This indicated that Pythium sp. can cause severe damage to safflower plants at 28° but not at 29° C.

There was a drop in survival of the two varieties in some of the pots at 25° and 30° C. Isolations made from pieces of lesioned safflower roots from these pots yielded a high percentage of an Oospora sp. The Oospora sp. has not, as yet, been proven pathogenic to safflower seedlings. No isolates of Pythium sp. were obtained from lesioned roots in these control pots.

Previous Culture Medium

Pythium sp. isolates A, B and C were grown for one week on lima bean sucrose and potato sucrose agar. The resulting growth was used to infest the soil in pots immediately after they were seeded with safflower varieties N. 3 and N. 9. Table 13 shows percentage survival of the two varieties three weeks after infesting the soil with the three isolates of Pythium sp. Fig. 9 shows the effect of the three isolates on safflower varieties N.3 and N. 9.

TABLE 13

Survival of safflower plants in soil infested with Pythium sp. grown on two agar media.

Medium, sucrose agar	Isol.	Percent survival		Survival, log 10 (x + 1.5)	
		N. 3	N. 9	N. 3*	N. 9*
Potato	A	23.1	0.6	0.4945	0.1946
	B	37.7	7.1	0.7230	0.3266
	C	16.7	0.2	0.4675	0.1853
Lima bean	A	18.3	0	0.4502	0.1761
	B	23.8	1.6	0.5363	0.2067
	C	10.8	0	0.3611	0.1761
Control		78.1	71.9		

* F (varieties) = 61.597
L. S. D. (interaction) = 0.1103

Survival of the two safflower varieties in soil infested with Pythium sp. differed significantly. Differences in survival of safflower in soil infested with Pythium sp. grown on the two media were not significant, nor were differences in survival of safflower in soil infested with the three isolates.

FIG. 9. Effect of three Pythium sp. isolates on the survival of two varieties of safflower. N. 3 is on the left and N. 9 is on the right of each pot. The treatments (left to right) are isolate A, isolate B, isolate C and control.

Host Species

Pots previously seeded with various crop plants were infested with isolate A of Pythium sp. An equivalent number of pots seeded with the various plants served as controls. Table 14 groups the plants according to their reaction to Pythium sp. The groups are as follows:

Highly susceptible - few plants emerged, roots of remaining plants severely lesioned or plants dead.

Susceptible - emergence decreased, roots of plants lesioned.

Resistant - emergence not decreased, roots of plants not lesioned.

None of the monocotyledons were susceptible to Pythium sp. Safflower and alfalfa were the only dicotyledons that were highly susceptible to the organism. The two clover species, sugar beet, cabbage and carrot were susceptible. These results indicate that Pythium sp. from safflower shows some specialization as to the higher plants which it can attack.

TABLE 14

Reaction of various crop plants in soil infested with *Pythium* sp.

Plant	Reaction
MONOCOTYLEDONS	
Wheat (*Triticum aestivum* L.)	resistant
Barley (*Hordeum vulgare* L.)	resistant
Oats (*Avena sativa* L.)	resistant
Rye (*Secale cereale* L.)	resistant
Corn (*Zea mays* L.)	resistant
Brome (*Bromus inermis* Leyss.)	resistant
Timothy (*Phleum pratense* L.)	resistant
Kentucky blue grass (*Poa pratensis* L.)	resistant
Crested wheat grass (*Agropyron cristatum* L.)	resistant
Onion (*Allium cepa* L.)	resistant
DICOTYLEDONS	
Pea (*Pisum sativum* L.)	resistant
Bean (*Phaseolus vulgaris* L.)	resistant
Field bean (*Phaseolus vulgaris* L.)	resistant
Cucumber (*Cucumis sativus* L.)	resistant
Flax (*Linum usitatissimum* L.)	resistant
Turnip (*Brassica rapa* L.)	resistant
Sugar beet (*Beta vulgaris* L.)	susceptible
Cabbage (*Brassica oleracea* L. var *capitata* L.)	susceptible
Carrot (*Daucus carota* L. var *sativa* DC.)	susceptible
Sweet clover (*Melilotus alba* Desr.)	susceptible
Alsike clover (*Trifolium hybridum* L.)	susceptible
Alfalfa (*Medicago sativa* L.)	highly susceptible
Safflower (*Carthamus tinctorius* L.)	highly susceptible

DISCUSSION

Pythium sp. showed a decrease in rate of growth with increasing time at 29° C. when grown on agar media containing extracts from plant tissues. Fawcett (15) found a similar decrease in the rate of growth with time, at near-maximum temperatures, in his studies on the effect of temperature on certain fungi. When Pythium sp. was cultured at 29° C. on sucrose nitrate agar the rate of growth, after an initial period of adjustment to conditions, exhibited no decrease with increasing time. Neither substituting various nitrogen sources for nitrate nor varying the amount of phosphate buffer in the sucrose nitrate agar caused the growth rate of the fungus to decrease with time at 29° C. The difference in the rate of growth of Pythium sp. at 29° C. on the various media did not appear to be related to the rate of growth of the fungus at other temperatures. Pythium sp. had a higher rate of growth at 20° and 25° C. on three of the four agar media which contained plant extracts than it had on sucrose nitrate agar. The other medium containing plant extracts supported a lower rate of growth by Pythium sp. than sucrose nitrate agar at 20° and 25° C. A possible hypothesis to explain this phenomenon is that the fungus when grown at near-maximum temperatures on media containing plant extracts accumulates a toxic product which inhibits growth. When the fungus is grown on the sucrose nitrate medium this toxic product is not accumulated in sufficient amounts to cause the growth retarding effect.

The carbon source and nitrogen source of the medium on which Pythium sp. was grown affected the rate of growth and amount of fruiting. However there was no apparent relationship between the rate of growth and the number of sporangia produced by the fungus on the carbon and nitrogen sources used in these studies. The presence of an hypha of the fungus is a prerequisite to the formation of a sporangium but the factors of the substratum which influence formation of sporangia are at least partially independent of those which govern the rate of linear growth.

The carbon and nitrogen sources of the medium on which Pythium sp. was grown affected the size of the sporangia produced by the fungus. These results suggest that the use of size and shape of sporangia as criteria for differentiating this Pythium sp. from other closely related species of Pythium should be avoided. Middleton (33) found the variation in size and shape of sporangia of various Pythium species was not sufficiently constant over a range of culture media to justify use of these characteristics as a means of differentiating species which produced spheroidal sporangia. Leonian (26) found that differences in the size and shape of the sporangia of various Phytophthora species was influenced more by the substratum on which the fungus grew than by the innate constitution of the fungus.

Pythium sp. was found to attack safflower in sterilized soil at temperatures from 5° to almost 30° C. The minimum and maximum temperatures for growth of the fungus were approximately 5° and 29° C. These results suggest that Pythium sp. is pathogenic to safflower at all temperatures at which it can grow. Erwin (14) found that Phytophthora drechsleri was able to attack safflower plants only at temperatures above 17° C. Pythium sp. is apparently able to attack safflower plants at lower temperatures than is Phytophthora drechsleri when the plants are grown in sterilized soil.

Pathogenicity tests indicate that safflower variety N. 3 is more resistant to attack by Pythium sp. than is N. 9. The reaction of the two varieties under field conditions (8) is similar to that found in the greenhouse. Greenhouse tests of safflower breeding material for resistance to Pythium sp. should give similar results to field tests. A method in which the soil is infested with Pythium sp. ten to 20 days after seeding would be expected to give more clearly defined results than one in which the soil is infested at the time of seeding.

SUMMARY

1. Pythium sp. grew at temperatures from 5° to 29° C. with maximum growth between 20° and 25° C.

2. Pythium sp. ceased growth one to three days following transfer to 29° C. when grown on agar media containing plant extracts but not when grown on sucrose nitrate agar.

3. Changes in the nitrogen source and buffer capacity of sucrose nitrate agar did not cause growth to decrease with time.

4. Nitrate, ammonium and amino sources of nitrogen supported growth of Pythium sp. The inorganic and protein sources, except ammonium acetate supported a higher rate of growth than the three amino acid sources. Cysteine supported very slow growth of the fungus.

5. All carbohydrates tested, except pectin, supported some growth of the fungus. The oligosaccharides and polysaccharides supported a higher rate of growth than the monosaccharides. Pythium sp. grew very slowly on xylose, D-arabinose, manose and fructose. L-arabinose and glucose supported reasonably good growth of the fungus.

6. Pythium sp. produced some sporangia on all media examined except one containing cysteine.

7. The number of sporangia and the size of sporangia produced by the fungus were influenced by the carbohydrate source and the nitrogen source of the medium.

8. There was no apparent relationship between the number of sporangia produced and the rate of growth of the fungus on various nitrogen and carbohydrate sources.

9. Three isolates of Pythium sp. produced sporangia at temperatures from 10° to 25° C. One of the three isolates produced a few sporangia at 5° C. The sporangia produced at 10° were smaller than those produced at 15°, 20° and 25° C.

10. Pythium sp. produced oospores with regularity only on a medium containing an extract of lima bean and on one containing wheat roots.

11. Two isolates of Pythium sp. produced oospores at temperatures from 10° to 25° C. A third isolate produced oospores only at 15°, 20° and 25° C.

12. Sporangia of Pythium sp. appeared to require water plus some other factor found in tap water and soil extract for direct germination. A period of below freezing temperature plus a substance present in safflower roots were found necessary to induce indirect germination of sporangia.

13. Safflower variety N. 3 had a higher percentage survival in soil infested with Pythium sp. ten and 20 days after seeding than it did when the soil was infested immediately after seeding. Variety N. 9 was equally susceptible to attack by Pythium sp. when the soil was infested at the time of seeding, ten days, or 20 days after seeding.

14. At soil temperatures from 5° to nearly 30° C. N. 3 showed a higher percentage survival than N. 9 in the presence of Pythium sp. Differences in the survival of the two varieties at the various temperatures were greater when the plants were grown for two weeks in the greenhouse before the soil was infested with Pythium sp.

15. Differences in survival of N. 3 and N. 9 in soil infested with Pythium sp. were similar to survival of the two varieties under field conditions.

16. Alfalfa, alsike clover, sweet clover, sugar beet and carrot as well as safflower were susceptible to attack by Pythium sp. in sterilized soil. Wheat, barley, oats, rye, corn, brome, timothy, kentucky blue grass, crested wheat grass, onion, pea, bean, field bean, cucumber, flax and turnip were unaffected by Pythium sp.

17. Cultures of Pythium sp. grown on potato sucrose agar and lima bean sucrose agar did not differ significantly from each other in pathogenicity to safflower seedlings in sterilized soil.

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APPENDIX I

Components of the various agar media used in the foregoing studies.

Potato Dextrose Agar

Washed sliced potato *	300 gm.
Dextrose	20 gm.
Agar	20 gm.
Distilled water	1000 ml.

Potato Sucrose Agar

The same as potato dextrose agar except sucrose substituted, in equivalent amount, for dextrose.

Lima Bean Sucrose Agar

Lima Bean (quick frozen) *	150 gm.
Sucrose	20 gm.
Agar	20 gm.
Distilled water	1000 ml.

Turnip Sucrose Agar

Turnip root *	300 gm.
Sucrose	20 gm.
Agar	20 gm.
Distilled water	1000 ml.

Water Agar

Tap water	1000 ml.
Agar	20 gm.

Wheat-Root Agar

Two or three root tips from recently germinated wheat seedlings added to 15 ml. of water agar before sterilization.

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* The requisite amount of plant material was boiled for 30 minutes in 500 ml. distilled water, filtered, and the resulting solution brought up to original volume. This solution was then mixed with the remaining 500 ml. of distilled water to which the carbohydrate and agar had been added.

Cornmeal Agar

Difco* prepared cornmeal agar	19 gm.
Agar	5 gm.
Distilled water	1000 ml.

Sucrose Nitrate Agar

Solutions of 0.1 M. potassium phosphate (KH_2PO_4), Magnesium sulphate ($MgSO_4.7H_2O$), sodium nitrate ($NaNO_3$), calcium nitrate ($Ca(NO_3)_2$), potassium chloride (KCl), and 0.01 M. solution of ferrous sulphate ($FeSO_4.7H_2O$) were made up with distilled water. The medium is as follows:

Potassium phosphate	25 ml.
Magnesium sulphate	5 ml.
Potassium chloride	25 ml.
Sodium nitrate	25 ml.
Calcium nitrate	5 ml.
Ferrous sulphate	2.5 ml.
Sucrose	20 gm.
Distilled water	to 1000 ml.
Agar	20 gm.

Safflower Sucrose Agar

Safflower root **	100 gm.
Sucrose	20 gm.
Distilled water	1000 ml.

Safflower Sucrose Nitrate Agar

100 gm. fresh safflower root ** added to sucrose nitrate agar.

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* From Difco Laboratories, Inc. Detroit, Michigan.

** 100 gm. safflower root triturated with 500 ml. distilled water in a Waring blendor for two minutes, centrifuged and the supernatent decanted off for use.